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Diurnal and Seasonal Variations of Plasma Corticosterone and Locomotor Activity in the White-Throated Sparrow, Zonotrichia Albicollis.

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Diurnal and seasonal variations of plasma corticosterone and locomotor activity in the white-throated sparrow, *Zonotrichia albicollis*

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Abstract

Studies in mammals have suggested a regulatory role for corticosterone on locomotor activity. Further, recent studies of the white-throated sparrow have indicated that the time of the diurnal release of hormones is important in regulating the physiological and behavioral events in its annual cycle. Therefore, a study was performed to ascertain whether the diurnal rhythm of plasma corticosterone levels shifted in accordance with the seasonal changes in the daily pattern of locomotor activity in the white-throated sparrow.

Plasma levels of corticosterone were measured fluorometrically at four times of the day on February 9 (winter), April 5 (prenuptial molt), May 5 and 15 (spring migration) and August 7 (postnuptial molt). A diurnal rhythm of plasma corticosterone levels occurs at each time of year. Moreover, the phase of the rhythm with respect to the photoperiod is different at each of the four seasons. Peak levels of corticosterone are not directly related to peaks of locomotor activity. Locomotor activity is better correlated with the disappearance of plasma corticosterone.

A seasonal variation in the absolute levels of plasma corticosterone also occurs. Corticosterone levels are highest in winter. They decrease linearly during the prenuptial molt, spring migration,

and postnuptial molt. A direct relationship between the seasonal levels of corticosterone in the plasma and the amount of locomotor activity does not occur. The total daily locomotor activity observed in May is over twice that which occurs in February, when plasma levels of corticosterone are highest.

Introduction

The finding of a diurnal rhythm of 17-ketosteroid levels in the urine of men was the first suggestion that the adrenal gland might have a diurnal rhythm of activity (Pincus, 1943). Because circulating levels of eosinophils are a good index of adrenocortical activity in mammals (for review, see: Halberg, Halberg, Barnum & Bittner, 1959), Halberg and his coworkers (1953) concluded on the basis of their studies of eosinophil levels that there was a diurnal rhythm of plasma adrenocortical hormones in mice.

A diurnal rhythm of plasma adrenocortical hormone was first demonstrated in the human (Doe et al., 1954; Tyler et al., 1954). In both studies, plasma 17-hydroxycorticosteroid levels were highest between 0600 and 0900 and lowest at 2200. A similar rhythm of 17-hydroxycorticosteroids was found in the monkey (Migeon et al., 1955). The corticoid levels were highest at 0600 and lowest at 2100. In both the human and the monkey the diurnal rhythms of adrenocortical hormone and circulating eosinophils are phased so that eosinopenia occurs at the time of increasing adrenocortical steroid levels.

The adrenocortical hormones are involved in the regulation of numerous physiological events. Their effects on levels of

blood glucose and liver glycogen and on gluconeogenesis, inflammation and immunity are well known. In addition, the adrenocortical hormones have been implicated in the regulation of body temperature, mitosis, locomotor activity (Halberg et al., 1958; Halberg, Peterson and Silber, 1959; Halberg et al., 1965) and sensitivity of the nervous system (Henkin et al., 1968; Frank et al., 1966; Woodbury et al., 1957; Woodbury, 1952). Most of these events have been shown to exhibit a diurnal rhythm of occurrence (for review, see: Sollberger, 1965).

Halberg has correlated the phase relationships of the diurnal rhythms of locomotor activity, liver glycogen, mitosis and body temperature with the diurnal rhythm of plasma adrenocortical hormone. While such correlations do not demonstrate causal relationships, Halberg suggests that the phase relationships of the adrenocortical hormones to physiological events is an important regulatory mechanism (Halberg, Halberg, Barnum and Bittner, 1959; Halberg et al., 1965). That is, the adrenals could impose a diurnal rhythm on various phenomena. Therefore if the adrenal exerts a regulatory influence on locomotor activity, it could be expected that the adrenal rhythm would have a specific phase relationship with respect to locomotor activity. In the mouse, the peak levels of plasma corticosterone precede the peak of locomotor activity by about four hours (Halberg, Peterson and Silber, 1959).

A role for the adrenocortical hormones in the regulation or maintenance of locomotor activity is supported by indirect

evidence. Halberg's findings that the diurnal rhythm of the adrenocortical hormones leads in phase the rhythm of locomotor activity is apparent in a variety of animals. The early morning peak of adrenocortical hormone usually occurs prior to the habitual time of awakening, or the beginning of daily locomotor activity in both the human and the Rhesus monkey. The human and the monkey are diurnal animals. In two other diurnal animals, the dog and the horse, plasma levels of adrenocortical hormones are highest at mid-morning (Harwood and Mason, 1956; Zolovik et al., 1966).

The rhythm of plasma adrenocortical hormones among nocturnal animals is considerably out of phase with the rhythms observed in diurnal animals. Plasma corticosterone in mice maintained on a 12-hour photoperiod is highest about two hours before the beginning of the dark period (Halberg, Peterson and Silber, 1959; Galicich et al., 1963; Haus, 1964).

A similar, but more variable, rhythm of corticosterone occurs in the plasma of the rat. Using a 14-hour photoperiod, Retiene and his co-workers (1968) found corticosterone levels to be highest about an hour before the beginning of the dark period. With 12-hour photoperiods, other workers (Saba, P. A. et al., 1965; McCarthy et al., 1960) found that plasma corticosterone was highest two hours after the beginning of the dark period. In another experiment, corticosteroid levels were highest shortly before sunset in rats exposed to a natural photoperiod of 11

hours on February 1 (Guillemin et al., 1959). An early evening peak of plasma adrenocortical hormones also occurs in the cat (Krieger and Krieger, 1967). In each of the animals listed above the diurnal rhythm of adrenocortical hormones leads in phase the diurnal rhythm of locomotor activity.

A well-defined diurnal rhythm of plasma corticosterone is not apparent in the nocturnal meadow vole (Seabloom, 1965). The levels of corticosterone appear to be higher in the late afternoon prior to increased levels of locomotor activity during the night. A biphasic rhythm of plasma cortisone (early morning and later afternoon peaks) is present in the catfish (Boehlke et al., 1966). Because these fish tend to be active both day and night, they cannot be classified as either diurnal or nocturnal animals. However, the peaks of cortisone seem to occur prior to the times that local fisherman report most active feeding. It is curious that the rhythm of plasma corticosterone in the catfish is out of phase with the rhythm of cortisone. Peak levels of corticosterone occur when cortisone levels are low.

A possible role of the adrenocortical hormones in the initiation or maintenance of locomotor activity possibly involves an effect on the nervous system. In normal men, there is a diurnal rhythm of total electroencephalogram (EEG) output with the greatest neural activity being at mid-morning (Frank et al., 1966). This EEG peak appears to lag by several hours the peak of plasma cortisol. The later afternoon troughs in the rhythms of both parameters have a similar phase relationship.

A causal relationship between the levels of adrenocortical hormone and EEG output is indicated by several experiments. Rabbits treated with dexamethasone, a synthetic glucocorticoid, exhibit increased behavioral alertmess and increased EEG activity (Shimada, 1966). There is an increase of the thalamic and reticular arousal responses as well. In humans an immediate increase in total EEG output follows injections of cortisol (Feldman et al., 1961).

The stimulation of neural activity by adrenal steroids could be a result of their effect on the distribution of ions in the nervous system. Woodbury and his co-workers (1957), using the electroshock seizure threshold (EST) as an index of the sensitivity of the nervous system, report that the glucocorticoids decrease the EST. That is, the glucocorticoids increase neural sensitivity. The increased sensitivity was correlated with an elevation in brain intracellular sodium and an increase in plasma potassium.

Cortisol and corticosterone were found to be concentrated up to 100 times higher in neural tissue than in the plasma of the human or the cat (Touchstone et al., 1966; Henkin et al., 1968). Following adrenalectomy of the cat, cortisol and corticosterone concentrations were disproportionately lower in the brain, spinal cord and the sciatic nerve compared to serum levels (Henkin et al., 1968). These responses were accompanied by an increase in serum potassium and perhaps a slight drop in serum sodium. Moreover, withdrawal of the adrenocortical hormones delays conduction across myoneural junctions and polysynaptic sensory systems (Ojemann and

Henkin, 1967; Chambers et al., 1963). These experiments suggest that the adrenocortical hormones play a significant though not as yet well-defined role in neural function.

More direct evidence for the involvement of the adrenals in locomotor activity is found in experiments on the migratory white-crowned sparrow (Meier et al., 1965). Injections of prednisone, a synthetic glucocorticoid, augmented nocturnal locomotor activity induced out of season by exogenous prolactin. This locomotor restlessness is regarded as an index of the physiological preparedness to migrate. An inhibition of migratory restlessness resulted from injections of metapirone, an inhibitor of glucocorticoid synthesis. The activity was restored, however, if the metapirone were accompanied by corticosterone. These experiments demonstrate that adrenocortical hormones are essential for an expression of migratory restlessness, but they do not indicate whether the hormones exert a causative effect or a permissive one.

In addition to a diurnal variation, adrenal glands also undergo seasonal variations in activity. Christian (1962) has described a biphasic annual rhythm of adrenal weight in the woodchuck. One peak occurs in spring and another in late summer. The peaks are associated with periods of increased social interaction, but reproduction seems to have no direct influence on adrenal weights. However, in the round-tailed ground squirrel, adrenal weights are highest during the spring reproductive period (Neal, 1965). The adrenal weights are lowest in September just prior to hibernation. Similarly, in a species of Australian tropical skink,

gross and histological measurements of the interrenal tissue indicate increased activity during the most active reproductive periods (Wilhoft, 1964).

Among birds, Hohn (1947) was unable to demonstrate any seasonal variation of adrenal weights in either the male or female mallard. But during the breeding season, the female showed a proportional increase in the cortical tissue over the medullary tissue, an increase not observed in the male. In a second study using more birds, Hohn and his co-workers (1965) found that adrenal weights of both male and female ducks increased during the breeding season and again in autumn and winter.

In other birds, histological criteria often provide conflicting information regarding the seasonal activity of the interrenal tissue. Burger (1938) observed histological changes indicative of increased cellular activity during the reproductive period of the non-migratory starling. Similar observations were made by Raitt (1968) on the non-migratory Gambel quail, and by Fromme-Bouman (1962) on the non-migratory European blackbird. Studies of migratory birds, however, do not support these results. Histological evidence suggests increased adrenal activity in Gambel's white-crowned sparrow during the winter, and decreased activity during the summer reproductive period (Lorenzen and Farner, 1964). John (1966) has shown histochemically that the content of adrenocortical hormone in the adrenals of two species of migratory Indian wagtails is higher during the migratory periods than the post-migratory periods.

Direct measurements of plasma adrenocortical hormone at different times of the year are few. Studies of two anadromous fish, the Pacific salmon and the migratory rainbow trout, have shown four-fold increases in the levels of plasma adrenocortical hormone during their migration from the sea to their freshwater spawning beds (Hane and Robertson, 1959; Robertson et al., 1961). There is also a marked hyperplasia of the adrenals. It is of interest to note that the non-migratory rainbow trout does not have adrenal hyperplasia nor an increase in plasma steroids prior to or during the reproductive period (Hane and Robertson, 1959).

Assenmacher and Boissin (1968) and Resko and co-workers (1964) have reported a seasonal difference in the levels of plasma corticosterone in the duck and the chicken. In both birds, plasma corticosterone levels were higher in winter than in spring. The significance of their results is not clear however, as neither study considered diurnal variations or the possibility that the phase of the rhythm might be different at different times of the year.

Because the adrenocortical hormones have been implicated in the regulation of several daily and seasonal events, it seemed of interest to learn whether the diurnal plasma levels of an adrenocortical hormone change phase from one season to another in accordance with shifts in various rhythmic phenomena. Accordingly, experiments were performed to determine whether there are parallel shifts in the diurnal rhythms of plasma corticosterone and locomotor activity at several seasons of the year. Corticosterone is the

primary steroid secreted by avian interrenal tissue (Nagra et al., 1960; deRoos, 1960, 1961). These experiments were performed on the migratory white-throated sparrow, Zonotrichia albicollis. This bird is especially well-suited for this type of study inasmuch as its temporal pattern of daily locomotor activity shifts from one season to another, having a nocturnal component during the migratory periods (Eyster, 1954).

Materials and Methods

The white-throated sparrow, Zonotrichia albicollis, breeds throughout much of southern Canada and northeastern United States, and winters primarily in the southern states and Texas (AOU Checklist, 1957). White-throated sparrows wintering in Baton Rouge, Louisiana, were caught with mist nets and traps from November - March in 1967 and 1968. The birds were housed in outdoor aviaries and maintained on a diet of chick starter crumbles, grain and water.

Blood for plasma corticosterone measurements was obtained by heart puncture from birds in four distinct physiological states: over-wintering (February 9), prenuptial molt (April 5), spring migration (May 5 and 15) and postnuptial molt (August 7). Plasma samples were obtained in each of these periods in 1967 and 1968. Sampling was done at six-hour intervals throughout the day beginning at sunrise. About one-half hour was required to complete the blood collections. The plasma was immediately collected and frozen at -15° C until the assays were performed. Plasma corticosterone is stable for many months if kept frozen (Guillemin, Clayton, Lipscomb and Smith, 1959). All assay birds were maintained in the outdoor aviaries for at least one month before use.

The concentration of plasma corticosterone was measured fluorometrically using an Aminco-Bowman Spectrophotofluorometer.

The procedures followed were those of Silber and his co-workers (1958) with the modifications for avian plasma of Nagra and his co-workers (1963).

One milliliter of plasma was added to a 50 ml screw cap centrifuge tube and diluted to 3.5 ml with deionized, glass-distilled water. Three and one-half milliliters of the standard solutions were also added to centrifuge tubes. To each sample and standard, 10 ml of petroleum ether was added, the mixture shaken for 30 seconds, centrifuged for 3 minutes and the petroleum ether layer (top) removed and discarded. This step was followed by an extraction with 10 ml of chloroform with the upper aqueous layer being discarded. To promote better protein precipitation and more thorough removal of lipids, cold chloroform (-10° C) was added slowly while swirling the centrifuge tube (Henry, 1964). The chloroform extract was shaken with 0.5 ml of 0.1 N NaOH for 15 seconds, centrifuged for 3 minutes and the NaOH (top) discarded. Because corticosterone is not stable in alkali, it is important to keep the exposure to NaOH to a minimum (Silber, et al., 1958).

Fluorescence was induced by adding 5 ml of the chloroform extract containing the corticosterone to 1.5 ml of a sulfuric acid: absolute ethanol mixture (65/35, V/V), also in a 50 ml screw cap centrifuge tube. This solution was shaken for 30 seconds, centrifuged for 2 minutes and the upper chloroform layer discarded. The time that the chloroform extract is added to the sulfuric acid: absolute ethanol mixture should be noted. The fluorescence was allowed to develop at room temperature for exactly 50 minutes,

at which time the samples were activated at 470 mu and the fluorescence read at 530 mu. The levels of plasma corticosterone were expressed as micrograms corticosterone per 100 ml plasma (ug%).

Corticosterone standards were prepared from a concentrated corticosterone stock solution. Corticosterone (Nutritional Biochemicals Corporation) was dissolved in absolute ethanol at a concentration of 4 mg/ ml and diluted to a concentration of 25 mg/liter with deionized glass-distilled water. A standard curve was obtained using corticosterone standards of 2.0, 1.5, 1.0 and 0.5 ug% (Figure 1). Deionized glass-distilled water was carried through the extraction procedure and served as a blank. During all extractions, it is important that the centrifuge caps be lined with an inert liner, such as teflon, because the chloroform and the activating medium extract a substance from the cap liner which fluoresces.

For each point determination it was necessary to pool the plasma from two and occasionally three birds. In 1968, only the plasma from consecutively killed birds was pooled to determine whether there was a difference between the plasma corticosterone levels of those birds killed first and those killed last.

Locomotor activity was recorded during the week prior to each of the sampling days. The activity cages were kept in an outdoor aviary exposed to the natural photoperiod. The birds were caged individually. Two perches in each cage were coupled to microswitches so that the depression of the perches resulted in the monitoring of locomotor activity on an Esterline-Angus

event recorder. Locomotor activity is expressed as an activity index (mean number of two minute intervals per hour with three or more hops).

Plasma levels of total lipids were measured on 50 ul plasma samples. One-half milliliter of a chloroform:methanol extraction mixture (2/1, V/V) was added to 50 ul of plasma in a 3 ml centrifuge tube. The mixture was stirred for one minute, centrifuged for three minutes and the chloroform:methanol phase (lower) containing the lipids removed with a syringe and saved. The remaining water phase and precipitate were similarly re-extracted twice and the chloroform:methanol phase from each of the extractions pooled. To the pooled mixture, one-fifth volume of 0.034% $MgCl_2$ was added to separate the chloroform and methanol. This mixture was stirred and centrifuged for 3 minutes, after which the upper methanol:$MgCl_2$ phase, along with any protein or lipoprotein flocculant, was removed and discarded. The remaining chloroform phase was transferred to a 1 dram screw cap vial and the chloroform evaporated over low heat (not over 37° C). The screw caps were lined with teflon. Following evaporation, 100 ul of chloroform was added from a micropipette and the vials tightly capped to avoid evaporation. Aliquots of 25 ul were transferred to tared electrobalance cups, the chloroform evaporated and the remaining lipids weighed. All weighings were made on a Cahn electrobalance. Three 25 ul replicates for each plasma sample were averaged and the results expressed in mg% plasma lipids.

The total body lipids of the assay birds were determined at each of the seasons studied. The carcasses were dried in a heated, vacuum desiccator. The body lipids were extracted with petroleum ether in Soxhlet apparati. Total body lipids are expressed as the dry lipid index (% lipid of dry body weight).

Statistical analyses of the diurnal and seasonal rhythms of plasma corticosterone were performed using the least-squares analysis of variance with the completely randomized design. Differences between the mean levels of plasma lipids were analyzed using Student's "t" test. All results are expressed as the mean $\pm$ the standard error.

Results

A. The Fluorometric Procedure.

Several of the methods for the determination of plasma corticosterone, including the one employed in this study (Nagra-Silber) have been criticized for their inability to remove interfering fluorogens (Frankel et al., 1967). Therefore several tests were performed to ascertain whether this assay could be used for plasma of the white-throated sparrow.

Fluorescence of a corticosterone standard develops gradually, reaching plateau levels in about an hour, remains at this level for approximately two hours and then begins a slow decay (Figure 2). Deviation from this fluorescence pattern could indicate the presence of an interfering fluorogen. The fluorescence intensities for plasma of the white-throated sparrow, pigeon and chicken were plotted over five hour periods and the curves compared to that of a 2 ug% corticosterone standard (Figure 2). The fluorescence curves obtained from the plasma of saline injected or untreated birds are essentially parallel to the corticosterone standard. This similarity indicates that little interfering fluorogen is present.

These results contrast from the fluorescence curve obtained by Frankel and his co-workers (1967) for chicken plasma using the

Nagra-Silber method. Their curve differed considerably from the corticosterone standard in that the fluorescence developed more slowly initially and was still increasing after three hours. Chicken plasma extracted by an extended fluorometric procedure designed by Frankel and his co-workers (1967), exhibited a fluorescence curve which paralleled the standard somewhat like our own results using the Nagra-Silber method. This extended method involves chromatographic purification of the steroid.

In a comparison of the Frankel extended method and the Nagra-Silber method using plasma from the adrenal vein of hypophysectomized, metapirone (inhibits glucocorticoid synthesis) treated chickens, Frankel found a large difference in the levels of plasma corticosterone. With his extended procedure, he measured 0.6 ug% corticosterone in the chicken. Using the Nagra-Silber method, the levels were 10.0 ug%. Frankel concluded that there is a residual contaminating fluorescence equivalent to about 10.0 ug% corticosterone when the Nagra-Silber method is employed. However, our data from the chicken and the white-throated sparrow demonstrate that residual fluorescence approaching this magnitude does not occur in our use of the Nagra-Silber technique. First, 50 minutes after activation, the mean fluorescence intensity of the plasma from the five chickens shown in Figure 2 is equivalent to 1.5 ug% corticosterone. This is considerably less than (about 1/6) the residual fluorescence alone reported by Frankel in the chicken using the same procedure. Second, the plasma levels

of corticosterone measured in the white-throated sparrow with this technique also fall well below the 10 ug% level (Table I). These values are among the lowest ever reported for plasma levels.

A possible source of fluorescence interference is plasma lipids (Frankel et al., 1967). Pigeons and white-throated sparrows were injected with estradiol benzoate to increase the plasma lipid levels (Riddle and Senum, 1939; Entenman et al., 1940) to test the effect of high plasma lipids on the fluorescence intensity. The pigeons received 2 ug estradiol/gram body weight and the white-throated sparrows 1 ug/gram body weight. Injections were made at the middle of an 8-hour (pigeon) or 12-hour (white-throated sparrow) photoperiod for three days. All birds in each experiment were killed at the same time of day. The estradiol injections increased total plasma lipid levels in the pigeon from 1176 to 4006 mg% ($p < .001$)(Table II). The increased plasma lipids are associated with an increase in plasma fluorescence which would be equivalent to a change in corticosterone from 1.9 to 8.0 ug% ($p < .001$)(Table II). Furthermore, the shape of the 5-hour fluorescence curve of the plasma from the estradiol treated pigeons varied markedly from that of the corticosterone standard and the saline injected pigeons (Figure 2).

In the white-throated sparrow, estradiol did not increase total plasma lipids. Plasma lipids were 536 mg% in the controls and 583 mg% in the estradiol treated birds (Table II). The fluorescence intensities of the plasma from the control and estradiol treated birds were equivalent to 2.6 and 2.5 ug%

corticosterone respectively. The difference is not significant (Table II). Moreover, the shape of the 5-hour fluorescence curves for these two groups of birds were the same (Figure 2).

These results suggest that in some cases, plasma lipids may interfere with the fluorescent determination of plasma corticosterone. However, this source of interference does not appear likely in the white-throated sparrow inasmuch as estradiol did not affect the plasma lipid levels or the fluorescence. In addition, none of the birds killed for assay were in the reproductive stage when blood lipid levels could be expected to be higher. However, these results must be interpreted with caution, as it is by no means clear how much the higher levels of plasma lipid may cause a false rise in plasma corticosterone inasmuch as estrogen may also stimulate an increase in adrenal activity (Kitay, 1963).

Care must be exercised to avoid contamination from other sources, such as color bands on pipettes, rubber particles (Frankel et al., 1967), or stopcock grease, protein precipitant and cap liners. Numerous interferences such as the above could provide a false high in the levels of corticosterone. Because our determinations of corticosterone levels are much lower than those of Frankel and his co-workers, their criticisms of the Nagra-Silber technique may be justified in the peculiar conditions of their experiments, but they do not appear valid in the context of our own experiences.

B. Seasonal Values of Body Weight, Body Fat, Gonad Weights and Locomotor Activity.

The seasonal variations of body weight in white-throated sparrows maintained in the outdoor aviaries coincided temporally with the seasonal fluctuations of body weight in feral birds (Figure 3). Total body lipids were determined on the assay birds at each of the four seasons studied. The increase in body weight of the white-throated sparrow during spring migration was a result of increased fat storage. At this time body lipids comprised 53.4% of the dry body weight (Table III). Total body lipids in February, April and August were much lower, being 19.0, 17.5 and 15.7%, respectively, of the dry body weight.

Gonadal stimulation was not evident among wintering birds or among birds during the prenuptial or postnuptial molt. The paired testes weights were between 2 and 3 mg at each of these seasons. Similarly, ovarian and oviducal weights were low at these times, being less than 13 and 7 mg, respectively. In May gonadal recrudescence was evident. Paired testes weights had increased to 46 mg. The ovaries (24 mg) and oviducts (11 mg) were also stimulated, but to a lesser extent. The female reproductive system responds more slowly to photoperiodic stimulation (Farner et al., 1966).

The prenuptial and postnuptial molts occurred among the aviary birds at the same time that they occurred in the feral populations. Compared to the time when the feral population of white-throated sparrows leave Baton Rouge (Lowery, 1955;

personal observations), the appearance of nocturnal migratory activity was delayed two to three weeks among aviary birds. Feral birds complete the northward migration by the first week of June. Caged birds, however, show migratory restlessness throughout the spring and summer until the photorefractory or postnuptial molt period in August.

The diurnal pattern of locomotor activity in the white-throated sparrow at different seasons is shown in Figure 10. A peak of locomotor activity occurs within an hour of sunrise. This peak is followed by a gradual decrease in locomotor activity during the remainder of the morning and during the early afternoon. A second, smaller peak of activity occurs in the late afternoon in April and August, but not in February and May. In May the late afternoon peak appears to be shifted into the night becoming nocturnal migratory restlessness. A well-defined second peak of activity does not occur in February. The phases of the rhythms of locomotor activity are different at different times of the year.

C. Diurnal and Seasonal Levels of Plasma Corticosterone.

The individual determinations of plasma corticosterone are summarized in Table I. In wintering birds on February 9, a diurnal variation of plasma corticosterone occurs. Throughout the day the levels of corticosterone increase $2\frac{1}{2}$ times from a low of 2.9 ug% at sunrise to a peak of 7.0 ug% shortly after sunset (Figure 4). During the night the levels fall. This rhythm, analyzed by

orthogonal comparisons (Table IV), shows a significant ($p < .05$) quadratic response and a highly significant ($p < .01$) cubic response. That is, the lines which best fit the rhythm are a parabola and sigmoid curve, respectively.

During the prenuptial molt (April 5) the phase of the rhythm of corticosterone differs from that found in wintering birds. Corticosterone is now highest six hours after sunrise (3.9 ug%) and lowest at sunset (2.9 ug%)(Figure 5). Further, the amplitude of the April rhythm is noticeably less than in February. The orthogonal comparison indicates that the differences between the peaks and troughs are significant (Table V). The response surface of the rhythm of corticosterone may be a parabola ($p < .05$) or a sigmoid curve ($p < .01$).

Figure 6 illustrates the levels of plasma corticosterone on May 5 and 15 during the spring migratory period. Once more, the phase of the diurnal rhythm differs from those of the previous seasons. Peak corticosterone levels (3.5%ug%) occur at sunrise and gradually disappear from the plasma reaching a low of 2.1 ug% at midnight. The decrease in plasma corticosterone is a highly significant linear effect ($p < .001$) although the rate of corticosterone disappearance appears to be greatest between sunset and midnight (Table VI).

During the postnuptial molt, plasma levels of corticosterone are high during the last half of the night (Figure 7). A peak of 3.7 ug% occurs at sunrise. The plasma levels decrease rapidly to a low of 2.1 ug% around noon. The levels remain low throughout

the rest of the daylight hours and increase during the early night to a value of 3.0 ug% at midnight. The rhythm is a highly significant parabola ($p < .01$)(Table VII).

It is apparent that the phase of the diurnal rhythm of corticosterone shifts from one season to another. The analysis of variance (Table VIII) indicates that the differences in the response surfaces of the four rhythms are highly significant ($p < .01$). Furthermore, the rhythms vary as a function of the time of year. That is, each of the four rhythms is associated with a particular physiological condition in the annual cycle of the white-throated sparrow. The phase relationships of these rhythms are presented graphically in Figure 8.

In addition to the seasonal differences with respect to the phases of the diurnal rhythms, there is also a seasonal variation in absolute levels of plasma corticosterone (Figure 9). The seasonal levels were determined by averaging the mean concentration of corticosterone at each of the four sampling times during the day. Corticosterone levels were highest during the winter. On February 9, the mean concentration during the day was 5.0 ug%. During the successive periods of the prenuptial molt, spring migration and postnuptial molt, there is a decrease in the levels of corticosterone in the plasma. The levels at these three seasons are 3.5, 3.0 and 2.7 ug%, respectively. The analysis of variance (Table VIII) indicates that this decrease is a highly significant ($p < .01$) linear effect.

The order in which the birds were killed at the different sampling times did not appear to influence the levels of plasma corticosterone. That is, our presence in the aviary did not induce a significantly greater release of corticosterone in those birds killed last than in those killed first.

Experiments in the rat have shown that there may be sex differences in the amplitude and phase of the diurnal rhythm of corticosterone. These differences have been attributed to the gonadal hormones (Retiene et al., 1968; Kitay et al., 1966; Kitay, 1963; Critchlow et al., 1961). Because the male and female white-throated sparrows are not distinguishable by external morphology and because the Baton Rouge population is only about 39% male, no attempt was made to pool the blood on the basis of sex. In those instances where pooled samples were from one sex, there was no apparent difference in the concentration of plasma corticosterone between males and females. Inasmuch as the birds were not reproductively active, influences by the gonadal hormones might be expected to have been minimal.

Discussion

An estimate of the seasonal adrenal activity in the white-throated sparrow was made by measuring the plasma levels of corticosterone. This estimation takes into account diurnal variations of the hormone. Plasma corticosterone levels decrease linearly ($p < .01$) in the white-throated sparrow from February to August (Figure 9). These results agree with the histological evidence of adrenal activity in the white-crowned sparrow, a migrant closely related to the white-throated sparrow (Lorenzen and Farner, 1964). Similarly, the studies of Assenmacher and Boissin (1968) and Resko and co-workers (1964) in the duck and the chicken also show higher levels of plasma corticosterone in winter than in spring.

Our results are not in agreement with those reported for the European blackbird, a non-migrant (Fromme-Bouman, 1962). In this bird adrenal activity, using histological criteria, was highest in the summer and lowest in late fall. The reason for this difference between migratory and non-migratory birds is not clear. It is noteworthy that in the white-throated sparrow there is no simple direct relationship between the seasonal levels of corticosterone in the plasma and the amount of locomotor activity. Whereas daily locomotor activity observed in May is over twice that which occurs in February, the levels of plasma corticosterone

are highest in February. Perhaps seasonal variations of hormone production are of lesser importance than diurnal variations.

The phase of the diurnal rhythm with respect to the photoperiod differs at various seasons of the year (Figure 8). There is a unique diurnal rhythm of corticosterone in the white-throated sparrow for the four seasons of the annual cycle. A least-squares analysis of variance (Table VIII) indicates that the differences in phase of the various corticosterone rhythms are the result of the effects of the time of year and not the effects of the time of day ($p < .01$). That is, there is no diurnal rhythm of plasma corticosterone if the levels of corticosterone at sunrise at all four seasons, as well as the levels of corticosterone at 6, 12, and 18 hours after sunrise for all four seasons, are averaged.

It is noteworthy that the phase of the specific diurnal rhythms of adrenocortical hormones reported for humans, the laboratory mouse and the laboratory rat are remarkably similar for each particular species. In view of the large number of studies of the diurnal rhythm of plasma adrenocortical hormones in these three animals, it seems highly unlikely that all the studies were performed at the same time of year. The reproducibility of results makes it appear that the phase of the diurnal rhythm of plasma adrenocortical hormones does not shift significantly throughout the year. The lack of a phase shift in these animals and the presence of phase shifts in the white-throated sparrow may be correlated with the fact that the human, the mouse and the rat are not truly seasonal animals, whereas the white-throated sparrow

has a well-defined annual cycle. In those seasonal animals where rhythms of plasma adrenocortical hormones have been described (meadow vole, channel catfish and horse), the studies have been performed at only one time of the year.

The events of the annual cycle of the white-throated sparrow would seem to be timed to occur in a functional and adaptively beneficial sequence. Because many of the physiological and behavioral phenomena have an endocrine basis, the diurnal production and release of hormones, shifting in phase through the annual cycle, could be instrumental in timing their sequential occurrences. While the levels of most hormones have been shown to exhibit diurnal rhythms, only recently have the possible implications of this type of variation been explored in relation to regulation of an animal's daily and annual cycles.

The rhythmic presence of a hormone might be expected to induce a rhythmic response to it. Lamond and Braden (1959) found a diurnal rhythm of response to pregnant mare serum (PMS) or human chorionic gonadotropin (HCG) in the mouse. The ovarian and uterine weights of those mice receiving PMS or HCG in the evening were significantly greater after 24-hours than in those mice similarly treated in the morning. They hypothesized that a diurnal rhythm of the release of another pituitary factor other than gonadotropins synergized with the exogenous PMS and HCG in the evening to stimulate the greater response.

More recently the concept of physiological time, as measured by diurnal rhythms, has attained greater significance in the light

of experiments with prolactin. These studies lucidly demonstrate that the time when prolactin is present is of utmost importance in determining the qualitative nature of the response to it. In the white-throated sparrow, prolactin injections at the middle of a 16-hour photoperiod induce a fattening response comparable to that observed in feral birds during the migratory period. However, a suppression of fat stores results when prolactin is injected at the beginning of the photoperiod (Meier and Davis, 1967). Similarly, a diurnal rhythm of locomotor response to prolactin occurs in the white-throated sparrow. Prolactin treatment at the middle or end of the day in April induces migratory activity, whereas prolactin given shortly after sunrise is completely ineffective (Meier, 1969).

The rhythmic induction of these two events is of special significance. The time when prolactin induces fattening and nocturnal migratory activity (two events characteristic of the spring migratory period) corresponds to the time when prolactin is released endogenously from the pituitary of the white-throated sparrow in May (spring migration). Furthermore, during the postnuptial molt in August, the white-throated sparrow is very lean and shows no migratory activity. At this time in the annual cycle endogenous prolactin is released during the last half of the night or early morning (Meier et al., 1969), the time when prolactin injections induced conditions similar to those found during the postnuptial molt. They concluded that the time of the diurnal release of prolactin was an important factor in the regulation

of the physiological and behavioral events in the annual cycle of the white-throated sparrow. These results support the concept that the time of release of hormones during the day plays an important role in the regulation of physiological conditions.

In Halberg's studies of the nocturnal mouse, peak plasma levels of corticosterone occur consistantly shortly before the beginning of the dark period of a 12-hour photoperiod, or 4-6 hours prior to the peak of locomotor activity. This same phase relationship is maintained after reversal of the photoperiod (Halberg et al., 1958). In the human, a predominantly diurnal animal, peak plasma levels of cortisol occur about 6-8 o'clock in the morning (Doe et al, 1954, 1960; Halberg, Halberg, Barnum and Bittner, 1959). In both mice and humans this peak of plasma adrenocortical hormone occurs when locomotor activity in usually minimal. Because corticosterone consistently led in phase the rhythm of locomotor activity, Halberg suggested that diurnal adrenal function did not constitute "responses to the stresses of daily life", as was believed, but rather that the adrenal was a "pacemaker" capable of phasing the rhythms of other physiological phenomena (Halberg, Halberg, Barnum and Bittner, 1959; Halberg, Peterson and Silber, 1959).

A similar phase relationship between plasma corticosterone and locomotor activity does not occur in the white-throated sparrow with respect to either the morning or afternoon peaks of activity (Figure 10). Instead the relationships are quite varied. Because the plasma levels of corticosterone do not necessarily reflect

directly the rhythm of adrenal secretion (Saba, G. C. et al., 1963; Saba, P. A. et al., 1965) these results do not necessarily refute the hypothesis of the adrenal's role in timing locomotor activity. Inasmuch as a fall in plasma steroid levels could mean an increased rate of utilization, the phase relationships of corticosterone and locomotor activity in May and August may be of special interest. The onset of nocturnal migratory activity in May and the onset of morning activity in August occur at times when the decrease of corticosterone levels in the plasma is greatest (Figure 10).

A specific time relationship between corticosterone and prolactin with respect to locomotor activity may be hypothesized. In May and August the diurnal release of prolactin from the pituitary (Meier et al., 1969) precedes the peak of locomotor activity and the time of the most rapid decrease of corticosterone from the plasma by approximately six hours (see Figure 10). Consideration of a possible functional integration of corticosterone, prolactin and locomotor activity in May and August raise several pertinent questions. First, the hormonal relationship does not correlate with the second, lesser daily activity peak present in April, May and August in the white-throated sparrow. Therefore, are there two types of activity in the white-throated sparrow, each determined by separate regulatory mechanisms? Second, is the morning activity peak during postnuptial molt (August) endocrinologically similar to spring nocturnal migratory activity but shifted 12 hours in phase? Third, what is the functional relationship between

corticosterone and locomotor activity in February and April? These questions emphasize that the role of corticosterone in the regulation of locomotor activity is not clear and that the data from the white-throated sparrow do not offer a ready explanation.

It is possible that corticosterone does not have a regulatory function on locomotor activity, but rather plays a permissive role. This concept of a permissive effect by a hormone was first proposed by Ingle (1952). A permissive effect by corticosterone would allow the white-throated sparrow to respond to the stimulus(i) for locomotor activity (e.g. prolactin) but not be able to induce the response alone. In the white-crowned sparrow, corticosterone augmented prolactin-induced nocturnal migratory activity. Alone, corticosterone was ineffective (Meier et al., 1965). That a certain level of corticosterone is required to "permit" the induction of locomotor activity is shown in a second experiment. Inhibition of corticosterone synthesis with metapirone blocked the inductive effect of prolactin on locomotor activity.

Early experiments indicate the need for at least permissive amounts of the adrenal steroids to maintain muscular work output. The spontaneous running activity of rats is reduced as much as 90% following adrenalectomy (Durrant, 1924; Richter, 1936; and Griffiths, 1949). Gans and Miley (1927) found that the total work performed by the gastrocnemius muscle of adrenalectomized rats was only 1/16 that of the intact controls. Complete muscle fatigue occurred within 10-60 minutes in these adrenalectomized animals, whereas contractions were sustained for 8-26 hours in

the controls. In intact dogs, injections of adrenocortical extract increased their running time and energy output 90-100% over their own normal base levels (Eagle et al., 1932). A mechanism to maintain sustained muscular activity has obvious advantages in a migratory bird.

In the mouse reversal of a 12-hour photoperiod caused a 180° phase shift in the rhythm of locomotor activity. The phase shift was accompanied by a corresponding shift in the rhythm of plasma corticosterone (Halberg et al., 1958). In the rat a 9-hour phase shift of a 14-hour photoperiod similarly shifted the rhythm of corticosterone in the plasma and the adrenal by nine hours (Critchlow, 1963). Similar experiments have not been done in the white-throated sparrow.

The seasonal changes of the diurnal rhythm of plasma corticosterone however, do not appear to be coupled directly to the photoperiod. From February 9 to May 15 the daily photoperiod increases from 11 to 13½ hours. During this time the phase of the diurnal rhythm of plasma corticosterone undergoes a 12-hour phase shift. In addition, the difference in the length of the photoperiod in May and August is only 15 minutes, but the response surface of the corticosterone levels at each of these seasons is very different. Similarly, although increasing photoperiods may stimulate diurnal shifts in locomotor activity (Wolfson, 1959), the seasonal changes are not directly related.

Shifting diurnal rhythms of corticosterone are important to the investigator. The dimension of time is an experimental

variable which can have a significant impact on the interpretation of experimental results. However, its significance has been largely overlooked. It is evident that if the comparison of experimental results is to be valid, it is imperative to ascertain the exact temporal conditions under which the data were obtained.

TABLE I.

Diurnal and Seasonal Levels of Plasma Corticosterone in the White-throated Sparrow.

February 9 (Winter).

0650 Hours[1]	1250 Hours	1850 Hours	0050 Hours
5.81[2]	4.48	4.20	2.48
4.23	9.80	7.10	7.15
1.26	0.87	5.12	9.80
2.17	5.30	7.55	2.31
1.75	2.34	10.42	5.71
2.34		7.00	
		7.70	
$\overline{2.92} \pm .73$[3]	$\overline{4.55} \pm .80$	$\overline{7.01} \pm .67$	$\overline{5.49} \pm .80$

April 5 (Prenuptial Molt).

0545 Hours	1145 Hours	1745 Hours	2345 Hours
2.45	2.48	1.61	2.83
4.13	2.21	3.01	5.80
3.57	3.22	4.02	3.01
3.68	4.10	1.26	3.32
3.64	3.25	2.52	3.72
3.78	3.32	4.55	3.74
3.43	5.25	3.60	
	5.32	2.66	
	5.71		
$\overline{3.52} \pm .67$	$\overline{3.87} \pm .59$	$\overline{2.90} \pm .63$	$\overline{3.73} \pm .73$

TABLE I. (Continued).

Diurnal and Seasonal Levels of Plasma Corticosterone in the White-throated Sparrow.

May 5 and 15 (Spring Migration).

0515 Hours	1115 Hours	1715 Hours	2315 Hours
0.70	3.01	1.57	0.17
1.50	7.00	2.80	0.17
1.40	4.90	0.88	2.52
2.31	2.17	0.60	3.43
1.33	2.55	7.35	2.41
6.30	2.27	3.88	2.91
7.66	.2.73	2.66	3.25
2.45	2.00	2.13	
2.31	3.74	2.87	
8.12		3.36	
2.83		5.77	
4.20			
5.04			
$\overline{3.55} \pm .49$	$\overline{3.37} \pm .59$	$\overline{3.07} \pm .53$	$\overline{2.12} \pm .67$

August 7 (Postnuptial Molt).

0530 Hours[1]	1130 Hours	1730 Hours	2330 Hours
2.06[2]	2.59	1.33	2.06
2.87	3.32	1.75	2.06
2.97	3.15	2.66	3.71
3.60	1.29	2.45	4.06
3.40	2.24	1.57	4.06
3.43	1.29	2.24	2.45
7.53	0.87	2.69	2.69
$\overline{3.69} \pm .67$	$\overline{2.10} \pm .67$	$\overline{2.09} \pm .67$	$\overline{3.01} \pm .67$

1. All Times are Central Standard Time.
2. ug% Plasma Corticosterone.
3. Mean $\pm$ Standard Error.

TABLE II.

Effects of Estradiol Benzoate on Total Plasma Lipids and Plasma Fluorescence Intensity in the White-throated Sparrow and the Pigeon.

	Plasma Lipids (mg%)		Fluorescence Intensity (ug% "corticosterone")	
	Control	Estradiol[1]	Control	Estradiol
White-throated Sparrow	466	608	2.8	3.4
	468	533	2.2	2.3
	597	498	2.8	2.1
	698	587	2.7	2.5
	453	690		2.4
	$\overline{536} \pm 45$[2]	$\overline{583} \pm 29$	$\overline{2.6} \pm .2$	$\overline{2.5} \pm .2$
Pigeon	1148	3776	1.8	7.4
	1280	4454	2.0	7.6
	1064	3672	1.8	8.6
	1222	4122		8.6
	$\overline{1176} \pm 46$	$\overline{4006} \pm 178$**	$\overline{1.9} \pm .1$	$\overline{8.0} \pm .3$**

1. Pigeons - 2 ug/gram body weight; White-throated Sparrows - 1 ug/gram body weight.

2. All results expressed as mean ± standard error.

** Significant at $p < .001$ compared to control.

TABLE III.

Seasonal Variations in Body Weight, Lipid Index, Gonadal Weights and Nocturnal Locomotor Activity in White-throated Sparrows Maintained in Outdoor Aviaries.

	Body Weight (grams)	Lipid Index (% Dry Weight)	Paired Testes (mg)	Ovary (mg)	Oviduct (mg)	NLA*
February 9, 1968 (Winter)	26.4	19.0	2.0	8.0	3.0	-
April 5, 1968 (Prenuptial Molt)	24.8	17.5	2.6	12.5	4.1	-
May 15, 1968 (Spring Migration)	30.5	53.4	46.0	23.8	10.7	+
August 7, 1968 (Postnuptial Molt)	26.2	15.7	3.0	11.0	7.0	-

* Nocturnal Locomotor Activity.

TABLE IV.

Analysis of variance and orthogonal comparison of plasma levels of corticosterone at four times of the day on February 9 (winter) in the white-throated sparrow.

Analysis of Variance

Source	df	SS	MS
Hours after sunrise	3	100.0	33.3
Error	19	82.3	4.3
Total	22	132.3	

Partition of time of day SS by use of orthogonal comparisons.

Effect	Hours after sunrise and ug% corticosterone				Q	Kr	SS	F
	0	6	12	18				
	2.92	4.55	7.01	5.49				
Linear	-3	-1	-1	-3	56.0	20(5.7)	18.4	4.27
Quadratic	-1	-1	-1	-1	26.9	4(5.7)	31.4	7.30*
Cubic	-1	-3	-3	-1	69.0	20(5.7)	41.3	9.62**
Total							91.1	

* $p < .05$
** $p < .01$

TABLE V.

Analysis of variance and orthogonal comparison of plasma levels of corticosterone at four times of the day on April 5 (prenuptial molt) in the white-throated sparrow.

Analysis of Variance

Source	df	SS	MS
Hours after sunrise	3	13.0	4.3
Error	26	20.1	0.8
Total	29	33.1	

Partition of time of day SS by use of orthogonal comparisons.

Effect	Hours after sunrise and ug% corticosterone							
	0 3.52	6 3.87	12 2.90	18 3.73				
Linear	-3	-1	-3	-1	16.3	20(7.5)	2.3	2.94
Quadratic	-1	-1	-1	-1	10.9	4(7.5)	4.0	5.20*
Cubic	-1	-3	-3	-1	28.6	20(7.5)	7.1	9.23**
Total							13.4	

* $p < .05$
** $p < .01$

TABLE VI.

Analysis of variance and orthogonal comparison of plasma levels of corticosterone at four times of the day on May 5 and 15 (spring migration) in the white-throated sparrow.

Analysis of variance

Source	df	SS	MS
Hours after sunrise	3	49.8	16.6
Error	36	108.3	3.0
Total	39	158.1	

Partition of time of day SS by use of orthogonal comparisons.

Effect	Hours after sunrise and ug% corticosterone				Q	Kr	SS	F
	0	6	12	18				
	3.55	3.37	3.07	2.12				
Linear	-3	-1	-1	-3	90.5	20(10)	40.9	13.6**
Quadratic	-1	-1	-1	-1	10.1	4(10)	2.61	0.9
Cubic	-1	-3	-3	-1	41.0	20(10)	8.40	2.8
Total							51.9	

** $p < .01$

TABLE VII.

Analysis of variance and orthogonal comparison of plasma levels of corticosterone at four times of the day on August 7 (postnuptial molt) in the white-throated sparrow.

Analysis of variance

Source	df	SS	MS
Hours after sunrise	3	12.6	4.2
Error	24	31.1	1.3
Total	27	43.7	

Partition of time of day SS by use of orthogonal comparisons.

Effect	Hours after sunrise and ug% corticosterone				Q	Kr	SS	F
	0	6	12	18				
	3.69	2.10	2.09	3.01				
Linear	-3	-1	-1	-3	14.4	20(7)	1.5	1.14
Quadratic	-1	-1	-1	-1	17.5	4(7)	10.9	8.40**
Cubic	-1	-3	-3	-1	4.6	20(7)	0.2	0.11
Total							12.6	

** $p < .01$

TABLE VIII.

Least-Squares Analysis of Variance: Diurnal and Seasonal Levels of Plasma Corticosterone in the White-throated Sparrow.

Source	d.f.	SS	MS	F
Year	3	75.28	25.10	7.86**
Linear	1	64.80	64.80	20.30**
Quadratic	1	9.37	9.37	2.93
Cubic	1	1.11	1.11	0.35
Day	3	2.21	0.74	0.23
Year x Day	9	82.53	9.17	2.87**
Error	105	335.09	3.19	
Total	120	1981.72		

** $p < .01$

Figure 1.

Corticosterone standard curve. The line was fitted by sight. RFI is the relative fluorescence intensity. The standards were prepared from crystalline corticosterone (Nutritional Biochemicals Corporation).

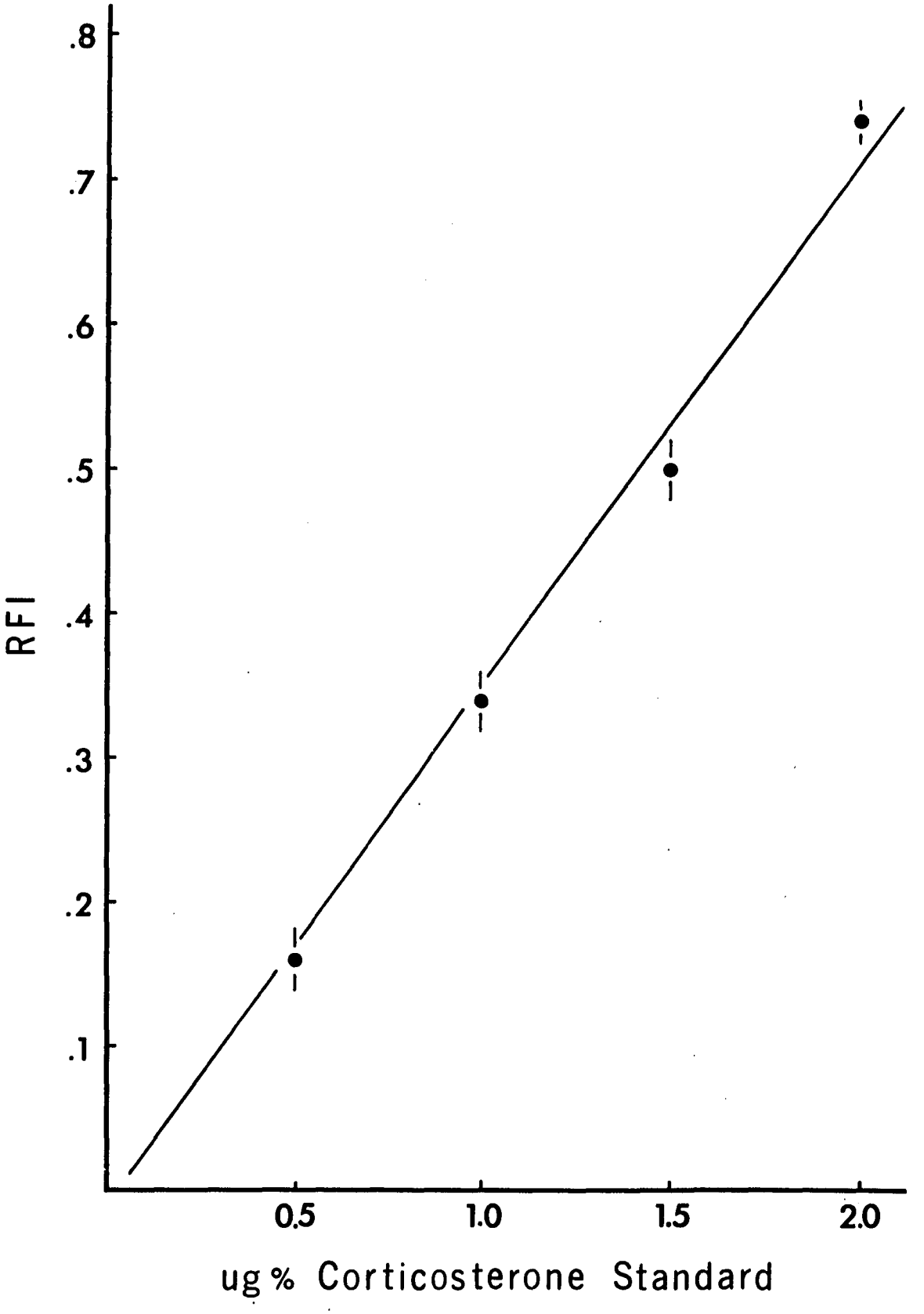
.8
.7
.6
.5
.4
.3
.2
.1
RFI
0.5
1.0
1.5
2.0
ug % Corticosterone Standard

Figure 2.

Temporal fluorescence pattern of a 2 ug% corticosterone standard and plasma from the white-throated sparrow, pigeon and chicken receiving various treatments. The numbers in parentheses are the number of birds in each group. RFI is the relative fluorescence intensity.

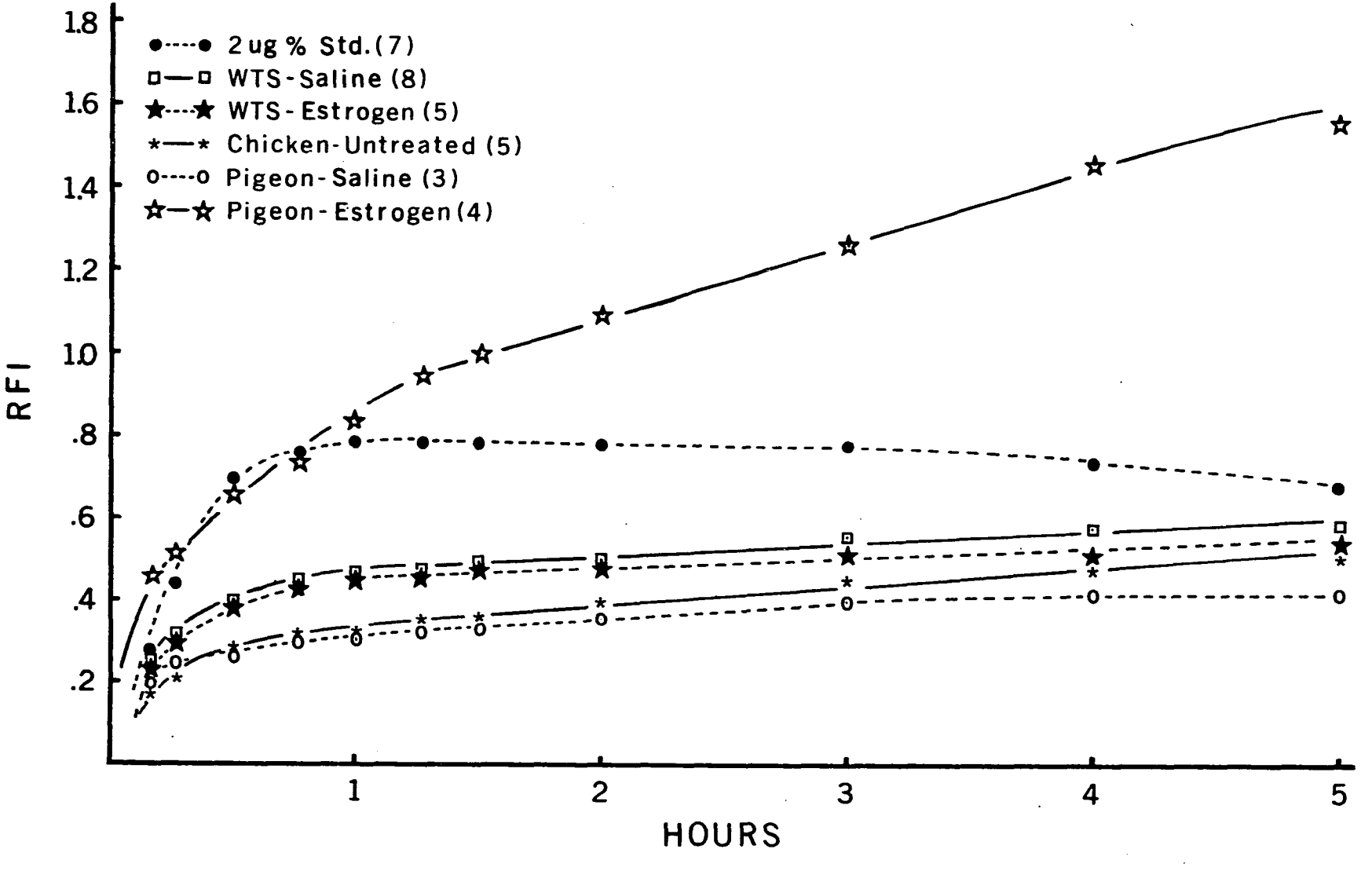
2 ug % Std. (7)
WTS - Saline (8)
WTS - Estrogen (5)
Chicken - Untreated (5)
Pigeon - Saline (3)
Pigeon - Estrogen (4)
RFI
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
1
2
3
4
5
HOURS

Figure 3.

Body lipid stores during the annual cycle of the white-throated sparrow in Baton Rouge, Louisiana. The values of birds which were killed immediately following capture (feral birds) are given as individual data. Mean values are provided for groups of birds (10-25) which were held in outdoor aviaries for at least one month before killing.

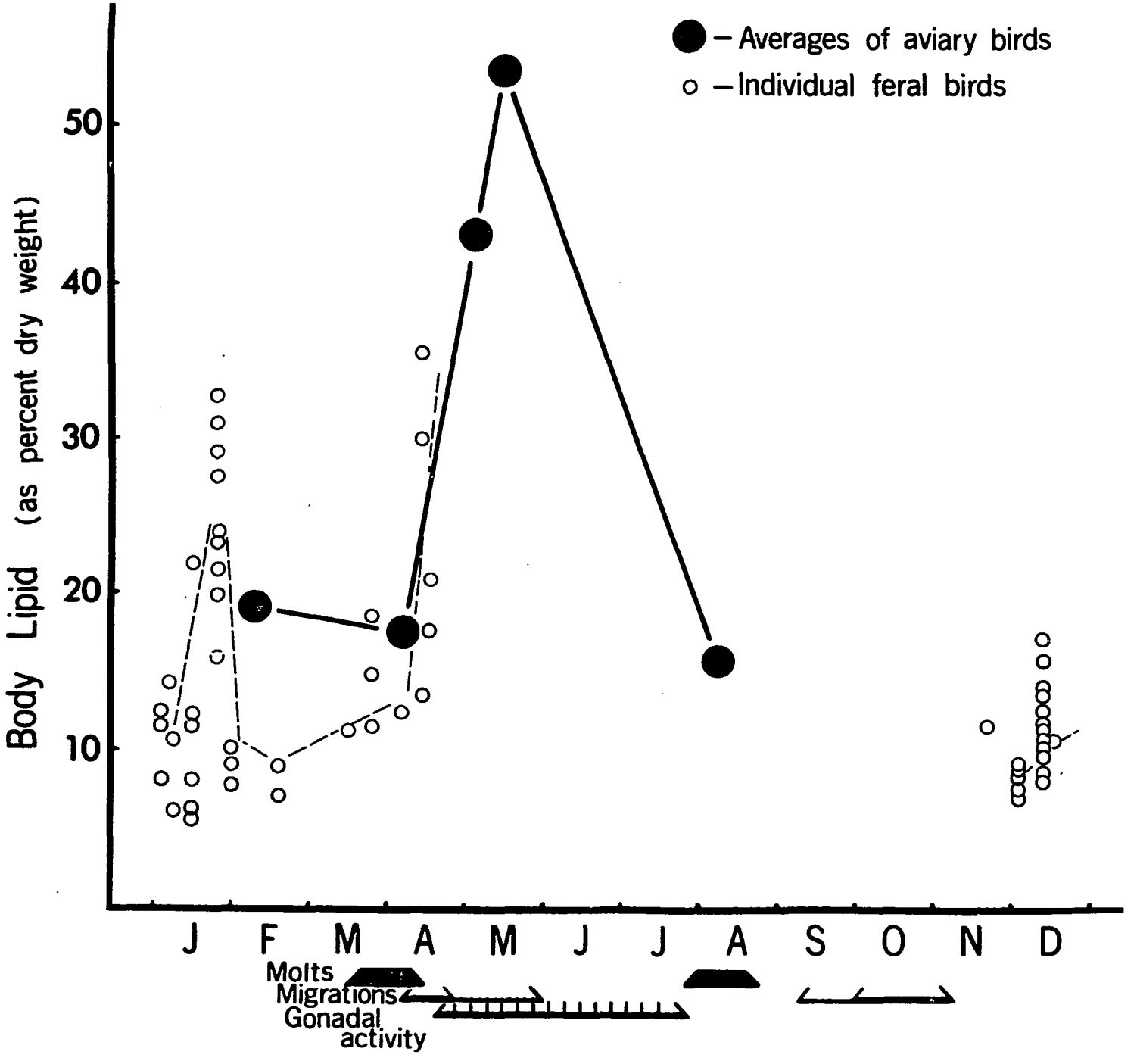
● – Averages of aviary birds
o – Individual feral birds
Body Lipid (as percent dry weight)
50
40
30
20
10
J
F
M
A
M
J
J
A
S
O
N
D
Molts
Migrations
Gonadal activity

Figure 4.

Diurnal rhythm of plasma corticosterone on February 9 in wintering white-throated sparrows. The values are expressed as the mean ± the standard error (SE). The natural photoperiod is indicated on the abscissa.

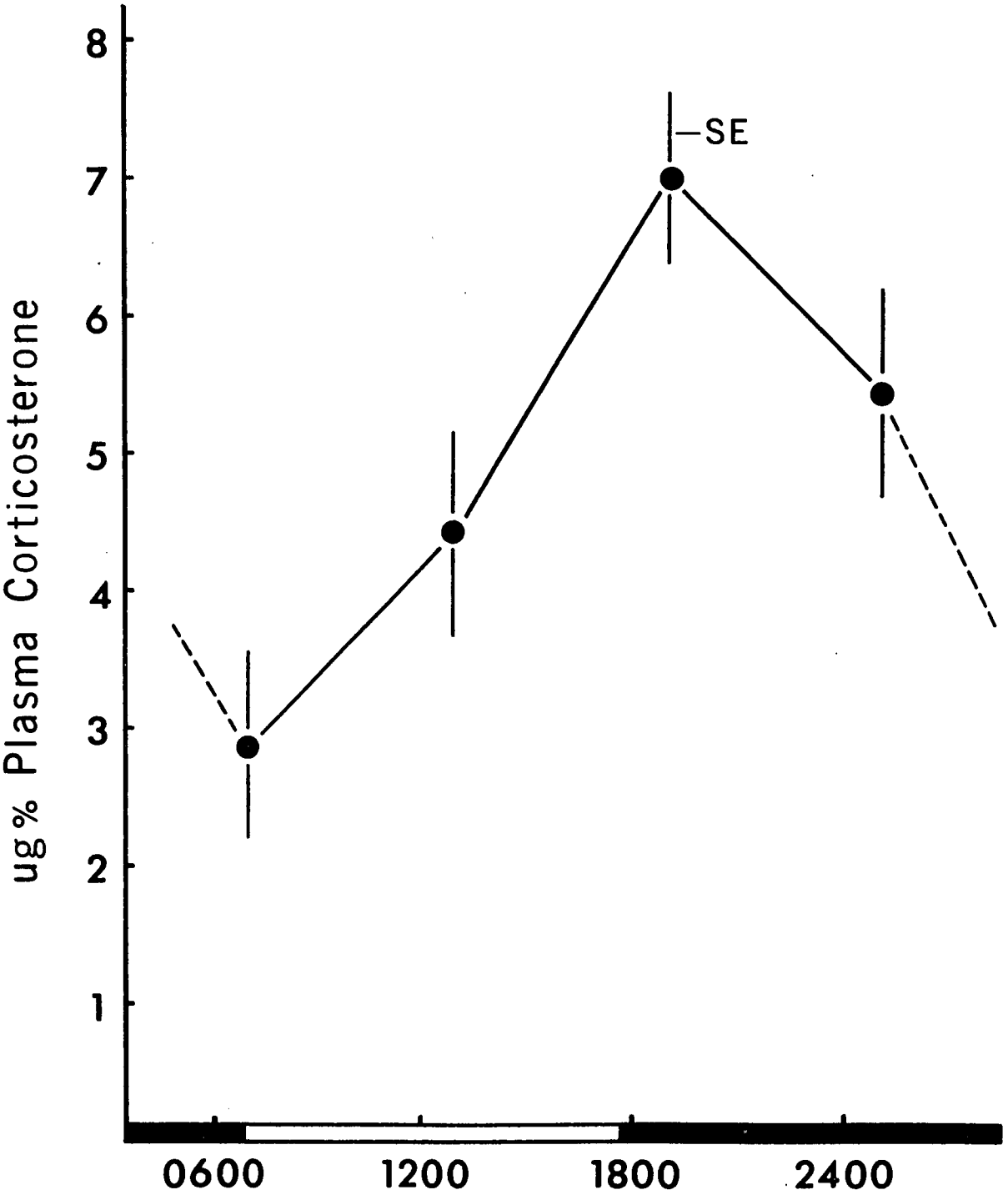
ug% Plasma Corticosterone
8
7
6
5
4
3
2
1
SE
0600
1200
1800
2400

Figure 5.

Diurnal rhythm of plasma corticosterone on April 5 during the prenuptial molt of the white-throated sparrow. The values are expressed as the mean ± the standard error (SE). The natural photoperiod is indicated on the abscissa.

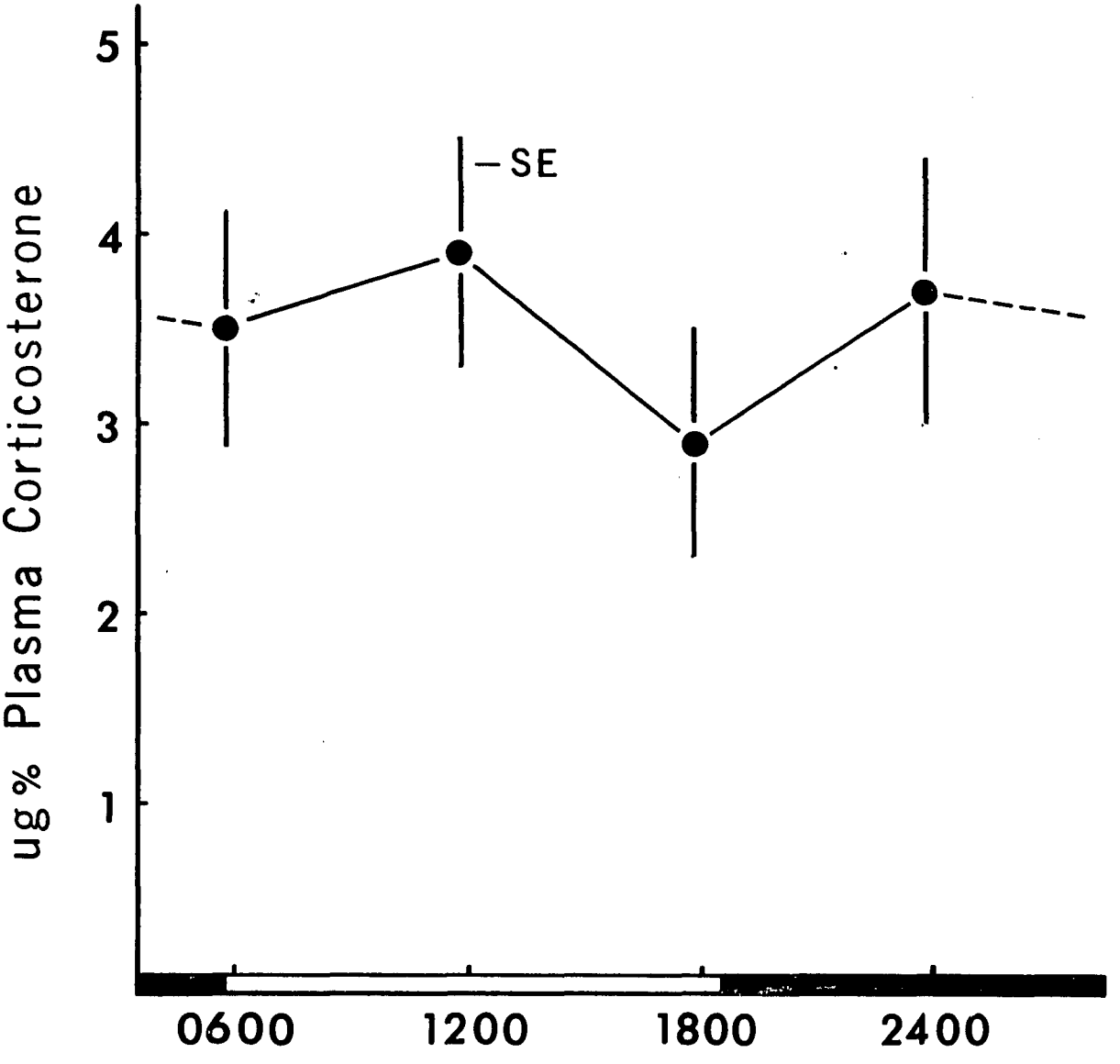
ug% Plasma Corticosterone
5
4
3
2
1
SE
0600
1200
1800
2400

Figure 6.

Diurnal rhythm of plasma corticosterone on May 5 and 15 in the white-throated sparrows in the spring migratory condition. The values are expressed as the mean ± the standard error (SE). The natural photoperiod is indicated on the abscissa.

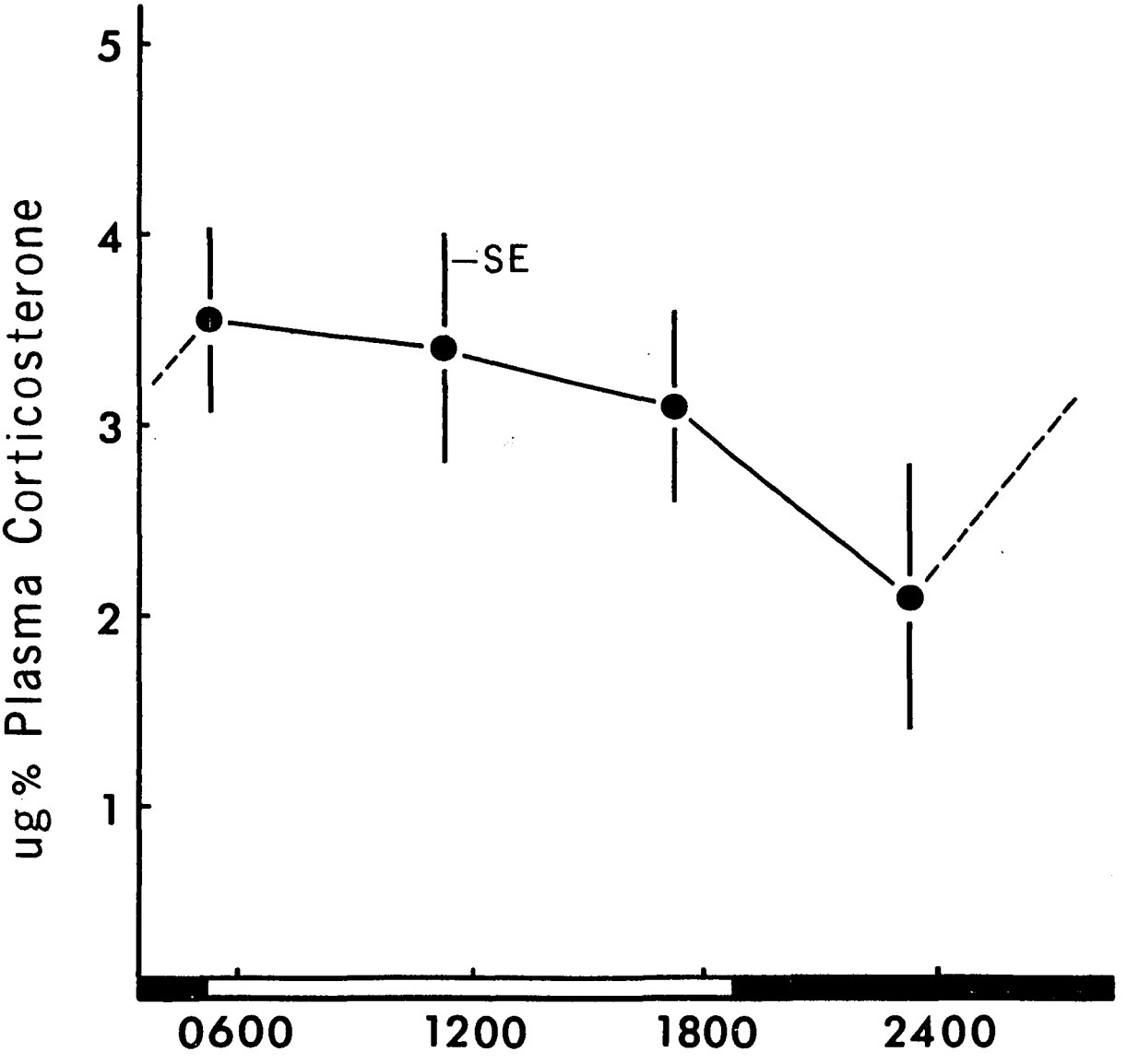
ug % Plasma Corticosterone
5
4
3
2
1
—SE
0600
1200
1800
2400

Figure 7.

Diurnal rhythm of plasma corticosterone on August 7 during the postnuptial molt of the white-throated sparrow. The values are expressed as the mean ± the standard error (SE). The natural photoperiod is indicated on the abscissa.

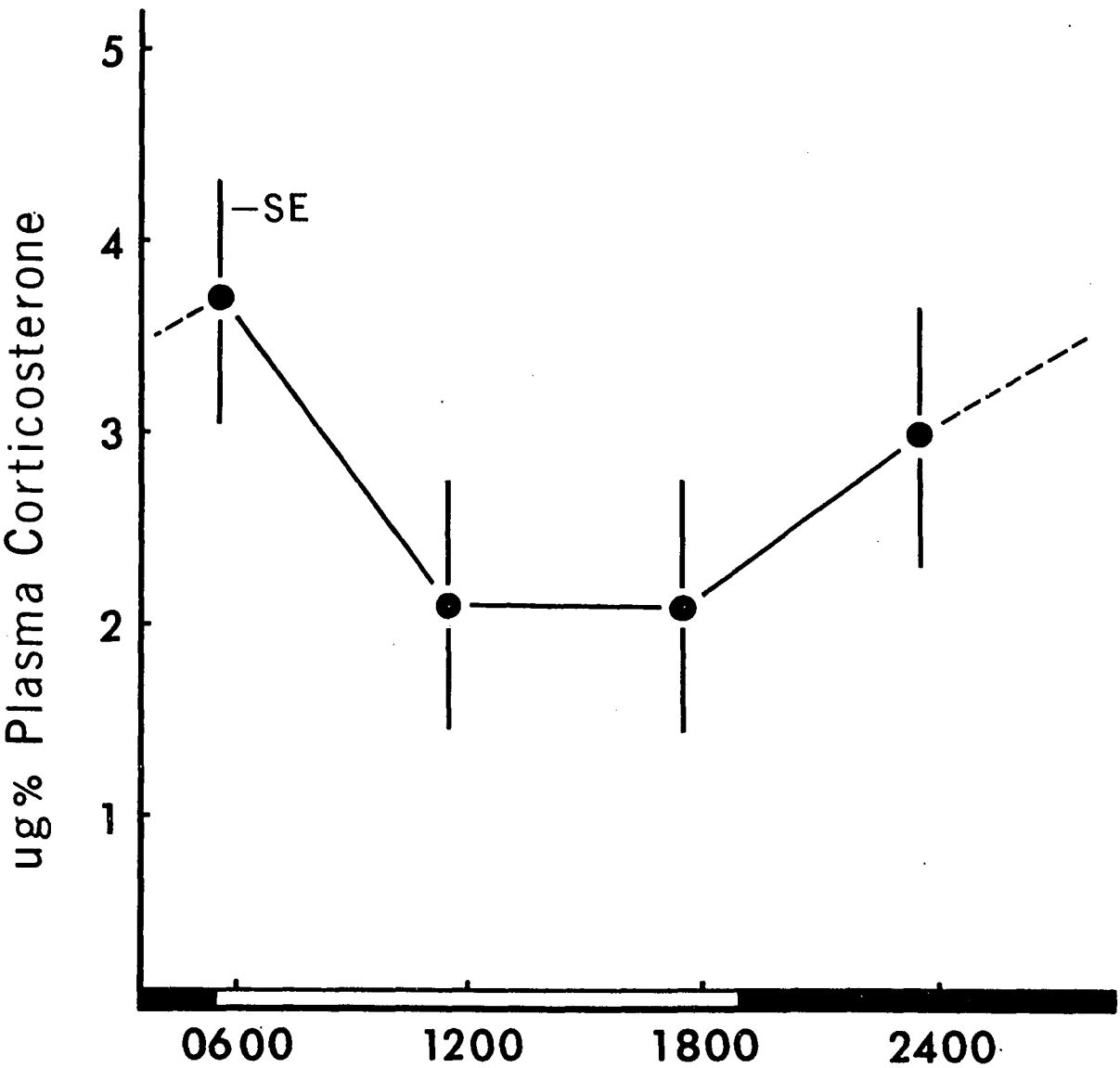
ug% Plasma Corticosterone
5
4
3
2
1
—SE
0600
1200
1800
2400

Figure 8.

Phase relationships of the diurnal rhythms of plasma corticosterone at four times during the annual cycle of the white-throated sparrow. February 9 - winter; April 5 - prenuptial molt; May 5 and 15 - spring migration; August 7 - postnuptial molt. Only the mean values of plasma corticosterone at the different times of day are given. The natural photoperiods at the different times of the year are indicated on the abscissa.

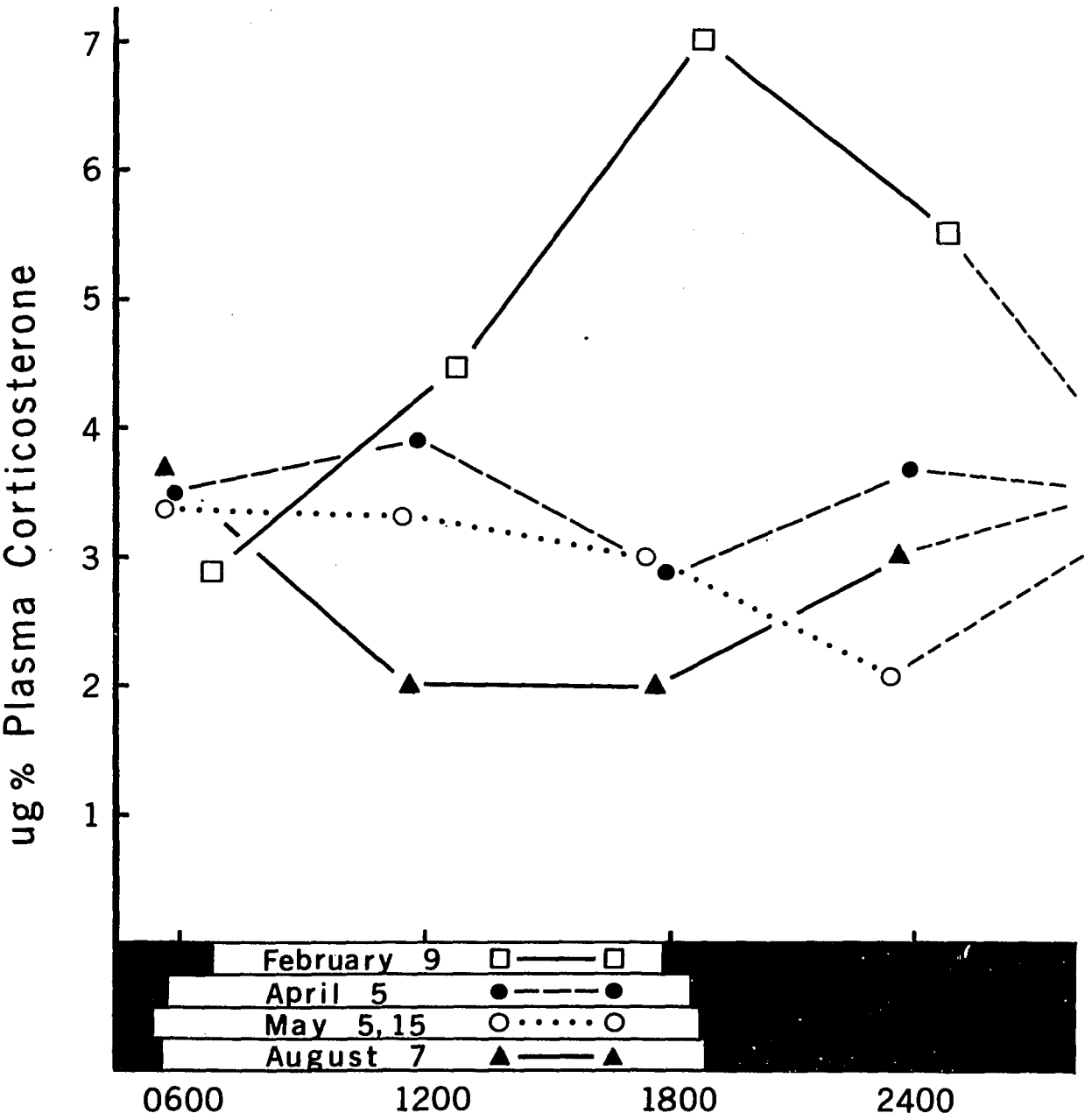
ug % Plasma Corticosterone
7
6
5
4
3
2
1
February 9
April 5
May 5, 15
August 7
0600
1200
1800
2400

Figure 9.

Seasonal levels of plasma corticosterone in the white-throated sparrow. The values were obtained by averaging the mean concentration of corticosterone at each of the four sampling times during the day.

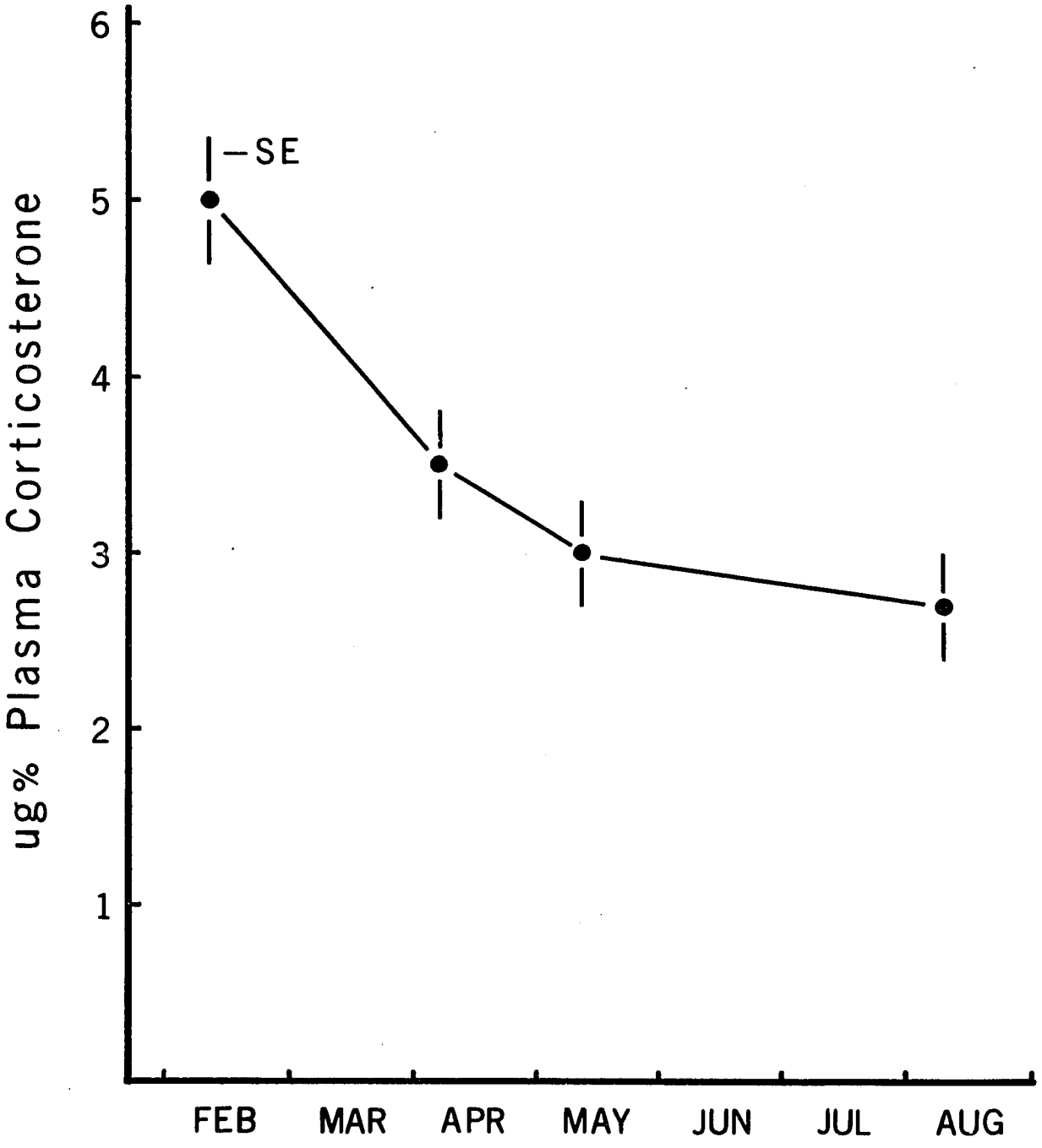
6
5
4
3
2
1
ug% Plasma Corticosterone
—SE
FEB
MAR
APR
MAY
JUN
JUL
AUG

Figure 10.

The phase relationships of the diurnal rhythms of plasma corticosterone (ACH), locomotor activity (L.A.) and pituitary prolactin (P) at four times during the annual cycle of the white-throated sparrow. The activity index is the mean number of 2 minute intervals per hour with 3 or more hops. Locomotor activity in February is the mean of 8 birds for 5 nights; in April, 4 birds for 5 nights; in May, 3 birds for 5 nights; and in August, 6 birds for 6 nights. Pituitary prolactin rhythms from Meier, Burns and Dusseau, 1969. Pituitary prolactin is expressed as the microgram equivalents of the standard (m.e.s.).

ug% Plasma Corticosterone
Activity Index
February
L.A.
ACH
April
May
P
August
Pituitary Prolactin (mes.)
0600
1200
1800

Summary

The temporal occurrence of the events in the annual cycle of white-throated sparrows maintained in outdoor aviaries is similar to that of feral birds. Prenuptial and postnuptial molt, spring migratory fattening and gonadal recrudescence occur among aviary birds at the same time that they occur among feral populations. The onset of nocturnal migratory activity, however, is delayed 2-3 weeks. This activity in caged birds persists throughout the spring and summer.

Plasma levels of corticosterone in the white-throated sparrow were measured fluorometrically at four times of the day on February 9 (winter), April 5 (prenuptial molt), May 5 and 15 (spring migration) and August 7 (postnuptial molt). A diurnal rhythm of plasma corticosterone levels occurs at each time of year. Moreover, the phase of the rhythm with respect to the photoperiod is different at each of the four seasons. That is, each diurnal rhythm is associated with a particular physiological condition in the annual cycle of the white-throated sparrow.

A seasonal variation in the absolute levels of plasma corticosterone also occurs. Corticosterone levels are highest in winter. They decrease linearly during the prenuptial molt, spring migration, and postnuptial molt.

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VITA

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EXAMINATION AND THESIS REPORT

Candidate: Jerry William Dusseau

Major Field: Vertebrate Zoology

Title of Thesis: Diurnal and Seasonal Variations of Plasma Corticosterone and Locomotor Activity in the White-throated Sparrow, Zonotrichia albicollis.

Approved:

Albert H. Meier
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Date of Examination:

July 3, 1969